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CLAIMS

What is claimed is:

1. A method for assaying a sample for an amplification product from a target polynucleotide, comprising:

contacting the sample which is suspected of containing the amplification product with a probe polynucleotide;

wherein the amplification product is a polynucleotide and comprises a capture sequence whose complement is not present in the unamplified target polynucleotide at the same position;

wherein the probe polynucleotide comprises a molecular beacon comprising first and second complementary regions and a third region located between the first and second complementary regions;

wherein the molecular beacon can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least part of the third region is complementary to at least part of the capture sequence in the amplification product, such that the probe polynucleotide can preferentially hybridize to the amplification product and thereby disrupt formation of the stem-loop structure under at least one set of hybridization conditions;

wherein the probe polynucleotide is linked to a first quencher and to a first fluorophore, wherein the first quencher and first fluorophore are located such that the first quencher can quench a fluorescence emission from the first fluorophore either under a first hybridization state when the probe polynucleotide is not hybridized to the amplification product and the stem-loop structure is formed or under a second hybridization state when the probe polynucleotide is hybridized to the amplification product and the stem-loop structure is not formed, but not under both hybridization states;

wherein the contacting takes place under conditions in which the probe polynucleotide can hybridize to the amplification product, if present;

exciting the first fluorophore by applying a light source to the sample; and

determining the fluorescence emission from the first fluorophore.

2. The method of claim 1, wherein the first fluorophore is a semiconductor nanocrystal.

3. The method of claim 2, wherein the semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, PbS, PbSe, an alloy thereof, and a mixture thereof.

4. The method of claim 3, wherein the core is CdSe.

5. The method of claim 1, wherein the first fluorophore is a dye.

6. The method of claim 5, wherein the dye is also the first quencher.

7. The method of claim 1, wherein the first quencher quenches the fluorescence emission from the first fluorophore under the first hybridization state.

8. The method of claim 1, wherein the first quencher quenches the fluorescence emission from the first fluorophore under the second hybridization state.

9. The method of claim 1, wherein the first quencher is selected from DABCYL, BHQ-1, BHQ-2, BHQ-3, a metal nanoparticle, and a semiconductor nanocrystal.

10. The method of claim 1, wherein the sample is assayed for the presence of the amplification product.

11. The method of claim 10, wherein a result of the assay for the presence of the amplification product is used to determine the presence of the target polynucleotide in the sample prior to production of the amplification product.

12. The method of claim 1, wherein the sample is assayed to determine the amount of the amplification product.

13. The method of claim 12, wherein a result of the assay to determine the amount of the amplification product is used to determine the amount of the target polynucleotide in the sample prior to production of the amplification product.

14. The method of claim 1, wherein the amplification product is produced at a detectably higher level from a first allele of a locus having at least two alleles.

15. The method of claim 14, wherein the at least two alleles differ by a single nucleotide.

16. The method of claim 14, wherein the first allele is an allele associated with an increased risk of a disease or disorder.

17. The method of claim 14, wherein the first allele comprises an inactivating mutation of a tumor suppressor gene.

18. The method of claim 14, wherein the first allele comprises an activating mutation of a cellular oncogene.

19. The method of claim 1, wherein the probe polynucleotide is attached to a solid substrate.

20. The method of claim 19, wherein the solid substrate is a microsphere.

21. The method of claim 20, wherein the microsphere comprises a spectral code comprising a first semiconductor nanocrystal having first fluorescence characteristics.

22. The method of claim 19, wherein the solid substrate is a chip.

23. The method of claim 19, wherein the solid substrate is glass.

24. The method of claim 19, wherein the solid substrate is a multiwell plate.

25. The method of claim 19, wherein the solid substrate is a fiber optic cable.

26. The method of claim 1, wherein the probe polynucleotide is attached to a porous gel matrix.

27. The method of claim 1, wherein the sample is simultaneously contacted with a second probe polynucleotide under conditions in which the second probe polynucleotide can hybridize to a second amplification product, if present, from a second target polynucleotide, wherein the second probe polynucleotide comprises a second molecular beacon and a second quencher and a second fluorophore having different fluorescence characteristics than the first fluorophore, wherein a fluorescence emission from the second fluorophore is quenched by the second quencher under only one hybridization state of the second probe polynucleotide with the second target polynucleotide, and wherein a second light source is applied to the sample to excite the second fluorophore, and further determining whether the fluorescence emission from the second fluorophore is quenched or unquenched.

28. The method of claim 27, wherein the first and second probe polynucleotides are attached to a solid surface.

29. The method of claim 28, wherein a single quencher comprises both the first and second quenchers.

30. The method of claim 27, wherein the first and second quenchers comprise the same quenching material.

31. The method of claim 27, wherein a single light source comprises both the first and second light sources.

32. The method of claim 27, wherein the first and second amplification products are produced from first and second alleles of a single locus.

33. The method of claim 32, wherein the first and second alleles are two alleles of a single nucleotide polymorphism.

34. The method of claim 32, wherein one of the first and second alleles is an inactivating mutation of a tumor suppressor gene.

35. The method of claim 32, wherein one of the first and second alleles is an activating mutation of a cellular oncogene.

36. The method of claim 32, wherein one of the first and second alleles is an allele associated with an increased risk of a disease or disorder.

37. A method of forming an amplification product detection complex for assaying a sample for a first target polynucleotide, comprising:

providing a first primer and a second primer;

said first primer comprising a 3' end, a first target complementary region that is complementary to the first target polynucleotide, said first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first target polynucleotide at a position 3' of a sequence to which the first target complementary region can hybridize;

said second primer comprising a 3' end;

providing the sample, said sample suspected of containing the first target polynucleotide;

contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first target polynucleotide and the first primer can be extended to form a first primer extension product;

altering the sample conditions to allow dissociation of the first primer extension product from the first target polynucleotide;

wherein the 3' end of the second primer is complementary to the first primer extension product at a position in the first primer extension product that is 3' to the first target complementary region;

contacting the sample with the second primer under conditions in which the second primer can hybridize to the first primer extension product and be extended to form a second primer extension product comprising a first capture sequence that is the complement of the first target noncomplementary region and does not exist elsewhere in the second primer extension product, wherein the second primer extension product is the amplification product;

altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product; and

contacting the sample with a probe polynucleotide comprising a molecular beacon comprising first and second complementary regions and a third region located between the first and second complementary regions, wherein the molecular beacon can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop, wherein at least a part of the third region is complementary to at least a part of the capture sequence, such that the probe polynucleotide can preferentially hybridize to the second primer extension product and thereby disrupt formation of the stem-loop structure under at least one set of hybridization conditions, and wherein said contacting takes place under said set of conditions, to form an amplification product detection complex;

wherein the probe polynucleotide is linked to a first quencher and to a first fluorophore, wherein the first quencher and first fluorophore are located such that the first quencher can quench a fluorescence emission from the first fluorophore either under a first hybridization state when the probe polynucleotide is not hybridized to the second primer extension product and the stem-loop structure is formed or under a second hybridization state when the probe polynucleotide is hybridized to the second primer extension product and the stem-loop structure is not formed, but not under both hybridization states;

exciting the first fluorophore by applying a light source to the amplification product detection complex; and

determining the fluorescence emission from the first fluorophore.

38. The method of claim 37, wherein the target polynucleotide is DNA.

39. The method of claim 37, wherein the target polynucleotide is RNA.

40. The method of claim 39, wherein a polymerase having reverse transcriptase activity is used to form the first primer extension product from the target polynucleotide.

41. The method of claim 37, wherein the target polynucleotide is single-stranded.

42. The method of claim 37, wherein the target polynucleotide is double-stranded.

43. The method of claim 37, wherein the sample is again contacted with the first and second primers after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product to form a plurality of first and second primer extension products.

44. The method of claim 37, wherein altering the sample conditions to allow dissociation of the first primer extension product from the target polynucleotide comprises raising the temperature of the sample.

45. The method of claim 37, wherein altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product comprises raising the temperature of the sample.

46. The method of claim 37, wherein the fluorescence emission from the first fluorophore is quenched under the first hybridization state.

47. The method of claim 37, wherein the fluorescence emission from the first fluorophore is quenched under the second hybridization state.

48. The method of claim 37, wherein the probe polynucleotide is present when the sample is contacted with the second primer.

49. The method of claim 37, wherein the fluorescence emission from the first fluorophore is monitored during formation of the second primer extension product.

50. The method of claim 37, wherein the fluorescence emission from the first fluorophore is monitored after formation of the second primer extension product.

51. The method of claim 37, further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product.

52. The method of claim 51, wherein removing single-stranded polynucleotides from the sample comprises adding a thermolabile single-stranded nuclease to the sample under conditions suitable and for a time sufficient to allow removal of single-stranded polynucleotides from the sample, and then heating the sample to inactivate the single-stranded nuclease.

53. The method of claim 37, wherein the target polynucleotide is a first allele of a locus having at least two alleles, and wherein the first primer preferentially is extended from the target polynucleotide as compared to another allele of the same locus.

54. The method of claim 53, wherein the first primer has at least one mismatch at one of the five 3' nucleotides of the first primer with said another allele.

55. The method of claim 53, wherein the at least two alleles differ by a single polynucleotide.

56. The method of claim 37, further comprising concurrently assaying the same sample for a second target polynucleotide by employing, in the same steps:

a second pair of first and second primers to form corresponding first and second primer extension products from the second target polynucleotide, wherein the first primer of the second pair comprises a second target noncomplementary region, wherein the second primer extension product formed from the second target polynucleotide comprises a second capture sequence complementary to the second target noncomplementary region;

a second probe polynucleotide comprising a second molecular beacon wherein at least a part of the third region thereof is complementary to at least a part of the second capture sequence and further

comprising a second fluorophore having detectably different emission characteristics from the first fluorophore and whose fluorescence emission is quenched in only one of the hybridization states of the second probe polynucleotide with the second primer extension product formed from the second target polynucleotide;

wherein the second probe polynucleotide can preferentially hybridize to the second primer extension product formed from the second target polynucleotide under said set of conditions;

and exciting the second fluorophore with the same or a different light source and determining if the fluorescence emission from the second fluorophore is quenched or unquenched.

57. The method of claim 56, wherein the first and second probe polynucleotides are linked and the first quencher can quench the fluorescence emissions from the first and second fluorophores independently based on the hybridization state of the first and second probe polynucleotides, respectively.

58. The method of claim 56, wherein the first and second probe polynucleotides are linked to a substrate that comprises the first quencher.

59. The method of claim 56, wherein the second probe polynucleotide comprises a second quencher, which may be the same as or different than the first quencher, wherein the second quencher quenches the fluorescence emission from the second fluorophore in only one of the hybridization states of the second probe polynucleotide.

60. The method of claim 56, wherein the first and second target polynucleotides are two alleles of the same locus.

61. The method of claim 60, wherein the two alleles differ by a single nucleotide.

62. An amplification product detection complex comprising a probe polynucleotide hybridized to an amplification product from a target polynucleotide, wherein the probe polynucleotide comprises a molecular beacon and the amplification product comprises a capture sequence not present in the target

polynucleotide, wherein at least a part of a loop region of the molecular beacon is hybridized to at least a part of the capture sequence, and wherein the molecular beacon comprises a fluorophore whose fluorescence emission is different than when the probe polynucleotide is not hybridized to the amplification product.

63. A kit for assaying for an amplification product from a target polynucleotide comprising:

a probe polynucleotide that comprises a molecular beacon comprising a stem-loop structure, a quencher, and a fluorophore;

a first primer comprising a 3' end, a first target complementary region that is complementary to the target polynucleotide, said first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the target polynucleotide at a position 3' of a sequence to which the first target complementary region can hybridize;

a second primer that is complementary to a first primer extension product produced by extension of the first primer when hybridized to the target polynucleotide, said second primer able to form a second primer extension product that is the amplification product from the first primer extension product;

a housing for retaining the probe polynucleotide, first primer, and second primer; and

instructions provided with said housing that describe how to use the components of the kit to assay a sample by forming an amplification product from the target polynucleotide using the first and second primers that comprises a capture sequence that is complementary to the target noncomplementary region in the first primer, wherein said capture sequence can bind to the loop of the molecular beacon and disrupt the stem-loop structure.